Characterization of Various Fast-Pyrolysis Bio-Oils by NMR Spectroscopy†

Charles A. Mullen,* Gary D. Strahan, and Akwasi A. Boateng

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 600 E. Mermaid Lane, Wyndmoor, Pennsylvania 19038

Received December 2, 2008. Revised Manuscript Received February 11, 2009

NMR spectroscopy, including ^{1}H, ^{13}C, and DEPT spectra, was used to characterize fast-pyrolysis oils from numerous energy crops and other agricultural feedstocks. The bio-oils studied were produced from switchgrass, alfalfa stems, corn stover, guayule (whole plant and latex-extracted bagasse), and chicken litter. The ^{1}H and ^{13}C NMR spectra were integrated over spectral regions to quantify classes of carbon and hydrogen atoms in each bio-oil sample. DEPT spectra were used to quantify by protonation, and the number of the carbon atoms in each of those classes was used to give further information on the types of molecules that are found in the bio-oil. The NMR spectra of the bio-oils varied greatly. The percentage of carbons and protons in the upfield regions of the NMR spectra tracked with the energy content of the bio-oil as well as the feedstock type, but there was no such consistent trend for the aromatic content. Degrees of branching in the aliphatic portions of the bio-oils were inferred from percentages of CH_1, CH_2, and CH_3 groups. Aromatic portions were found to be extremely complex, with substituted aromatic carbons outnumbering unsubstituted aromatic carbons >2:1 in most cases. Fully substituted carbons represented 27−37% of all the carbons in the sample.

Introduction

Fast pyrolysis of lignocellulosic biomass produces a dense liquid bio-oil in up to 70% yield.[1,2] As bio-oil is significantly more dense than its parent biomass, it can be more economically and efficiently transported to a centralized location for use as a feedstock for further processing, by gasification/Fischer−Tropsch synthesis, etc., to produce transportation fuels. However, bio-oil is a complex mixture of hundreds of oxygenated organic compounds that are the result of depolymerization of the three building blocks of biomass (cellulose, hemicellulose, and lignin), thus presenting stability and storage problems. Compounds found in bio-oils include acids, esters, alcohols, ketones, aldehydes, anhydrosugars, furans, and phenols. The exact composition of bio-oil depends on a number of factors, including biomass origin and composition, reactor temperatures, heating rates, residence times, and quench rates.[2]

Owing to its complex nature, chemical characterization of fast-pyrolysis bio-oils has been a challenging undertaking. GC analysis has often been used to identify and quantify individual compounds in bio-oil, but only 25−40% of bio-oil compounds are observable by GC methods because a large fraction of these oils consists of lignin and carbohydrate oligomers, which are not volatile enough to be observed by GC.[1,3] HPLC has been used to quantify some water-soluble species, and gel permeation chromatography (GPC) has also been used to obtain molecular weight distributions on higher molecular weight phenolic species derived from lignin,[4] but again this only characterizes a portion of the bio-oil. Classification of compounds has also been done using solubility by progressive extraction of bio-oil with various solvents.[4,5] Spectroscopic techniques like IR can give insights into functional groups present in the bio-oil, but only in a qualitative measure.[6,7] Some have used ^{1}H and ^{13}C NMR as a means of obtaining approximate ratios of the chemical environments of protons and carbon atoms[7−9] and determined the approximate aromatic:aliphatic ratios, but we know of no in-depth analysis of bio-oils using NMR that includes the relative substructure compositions. Unlike the other techniques men-

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* To whom correspondence should be addressed. Phone: 215-836-6916. Fax: 215-233-6559. E-mail: charles.mullen@ars.usda.gov.

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10.1021/ef801048b
Published on Web 04/15/2009

Table 1. Pyrolysis Conditions and Bio-Oil Properties

	switchgrass	corn stover	alfalfa stems	guayule (whole)	guayule bagasse	chicken litter
pyrolysis temperature (°C)[b]	480	503	483	478	479	488
% bio-oil collected at the ESP[c]	43	70	41	62	73	41
% bio-oil organics collected at the ESP[d]	47	85	67	64	75	49
C (wt %)	52.97	53.97	57.00	69.93	69.97	69.5
H (wt %)	6.43	6.92	7.89	8.54	7.96	8.23
N (wt %)	0.38	1.18	3.75	2.92	0.82	7.57
S (wt %)		<0.05	0.07	0.20	0.07	0.39
O (wt %)	39.13	37.94	31.30	19.31	21.38	13.87
H_2O (wt %)[e]	6.2	6.1	8.2	1.0	1.7	9.8
HHV (MJ/kg)	23.60	24.30	30.60	31.1	30.90	31.60

[a] Dry basis values of bio-oils collected at the ESP. [b] Average bed temperature during run; residence time in the bed is ~0.1 s. [c] Mass fraction of entire liquid product, including water collected at the ESP. [d] Mass fraction of liquid product, excluding water, collected at the ESP. [e] Determined by Karl Fischer titration.

tioned above, NMR has the potential of being able to examine nearly the entire, intact, bio-oil rather than a selected fraction of it.

We herein report an expanded and in-depth use of ^{1}H and ^{13}C NMR to characterize fast-pyrolysis bio-oils from numerous biomasses including switchgrass, alfalfa stems, guayule plant and bagasse, corn stover, and chicken litter, the production of bio-oil from which has been previously reported.[10−13] Additionally, ^{13}C DEPT (Distortionless Enhancement Polarization Transfer) spectra were integrated and used to quantify carbon atoms in the bio-oils on the basis of attached protons and chemical environment.[14,15] This method not only provides the percentages of carbons in different chemical functional groups (based on chemical shift), but also provides information on the substitution numbers of those carbon atoms. In this way, NMR can be used as an assay to determine which feedstocks and pyrolysis conditions produce bio-oil with particular desired characteristics (such as presence of functional groups, degrees of branching or saturation, aromatic content) for downstream production of fuels or chemicals.

Methods

Feedstocks. The bio-oils in this study were produced from six different feedstocks. They can be grouped into three categories: woodlike (chicken litter, guayule), legume (alfalfa stems), and grasslike (switchgrass, corn stover). Details on most feedstocks from which bio-oils in this study were produced have been previously published: switchgrass (cave-in-rock variety),[10] alfalfa stems (from plants harvested at full flower stage),[11] corn stover (stalks, leaves, and stems),[12] whole plant guayule and guayule bagasse (stems after latex extraction).[13] Chicken litter is a combination of hardwood bedding material (primary component) and chicken manure.

Pyrolysis, Bio-Oil Production. For all samples, the bio-oil was produced by fluidized-bed pyrolysis in silica sand medium at temperatures between 450 and 550 °C (Table 1), with residence times of ~0.1 s in the fluidized bed. Detailed descriptions of the pyrolysis system and conditions have been previously published.[10−13] The samples used for the NMR characterization were that fraction collected in the electrostatic precipitator (ESP), which is the last point in the condensation train, where most of the bio-oil is collected and moisture content is lowest. This ESP fraction represented 40−70% of the total liquid condensate (including water) collected and was picked for NMR analysis because of its homogeneity and low water content. The low water content had the benefit that the maximum amount of organic material was dissolved. While the organic fraction analyzed is likely to be similar to that of the entire organic liquid product, their relative concentrations are expected to be somewhat different.

Nuclear Magnetic Resonance Spectroscopy (NMR). Solution-state (acetone-d_6) NMR spectra were recorded at 9.4 T on a Varian Inova NMR spectrometer, using a 5 mm broad band probe. All experiments were performed at 40 °C, at which temperature the samples appeared to be well-dissolved but were nearly saturated. The ^{1}H (proton) spectra, at 400 MHz, were acquired with a 90° pulse angle and a 3 s relaxation delay; the sweep-width was 6000 Hz, and the sodium salt of 3-(trimethylsilyl)propionic acid-d_4 (TSP) was used as an internal reference. Suppression of residual water signal was not necessary in these samples as the water content in these fractions was very low, as noted above. All ^{13}C spectra were acquired at 100 MHz, with a sweep width of 30 000 Hz and a 90° pulse angle, and were referenced to the TSP peak, at 0 ppm. To allow for accurate integration of ^{13}C signals, the 1D ^{13}C spectra were recorded with broad band proton-decoupling only during the acquisition periods ("inverse-gating"), to avoid nonuniform enhancement of carbon signals from proton NOE effects (polarization transfer). As each sample was a complex mixture of hundreds of compounds, the concentration of each individual component was low even though nearly saturated solutions were used. These low concentrations, in combination with the intrinsic low sensitivity of the ^{13}C nuclei, required the use of 15 000−25 000 transients to achieve visually acceptable signal-to-noise ratios. Such lengthy signal-averaging made the direct determination of accurate carbon T_1 relaxation times impractical due to long acquisition times; hence, we could not be assured that we were using a delay of five times the longest T_1, which would have allowed the full recovery of all signals and the most accurate measurement of integrated intensities. Instead, a 6−8 s relaxation delay was used between scans, with no added relaxation agents, resulting in experiments with long acquisition times. Errors in the integrated intensities were estimated and mathematically corrected by use of a reference curve derived from intensities of a known standard mixture (vide infra). Fully edited DEPT experiments were run using the same parameters as the ^{13}C spectra, but with final flip angles of 45°, 90°, and 135° and an $^1J_{CH}$ coupling constant of 135 Hz, followed by mathematical manipulation to generate the DEPT subspectra for CH, CH_2, and CH_3.[16,17,20] For these experiments, 9000−11 000 transients were obtained for each of the four required spectra. As with the ^{13}C spectra, the

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integration values of the DEPT spectra needed adjustment for quantitative analysis. The DEPT subspectra were observed to have intensity distortions arising from both incomplete relaxation and the effects of $^1J_{CH}$ coupling. Mathematical correction of the integrated intensities was performed by reference to the results from the standard mixture, in accord with the observations and methods of others.[14,18,19]

The standard sample mixture consisted of equimolar amounts [1:1:1 (mol)] of ethyl benzene, 4-hydroxy-4-methyl-2-pentanone, and isobutyl alcohol and was analyzed using the same ^{13}C and DEPT parameters as described above. This relatively simple mixture was chosen because it provided a variety of common chemical functional groups with different proton numbers and covers most of the relevant chemical shift range. The individual spectra and subspectra were divided into smaller sections based on the chemical shift ranges that correspond to different chemical functional groups, and this was followed by integration. The integrated peak intensities (peak areas) in each section were then compared mathematically to their expected values to obtain a series of standard curves. These standard curves provided a measure of the approximate intensity errors, as well as correction factors, for carbons in different chemical shift ranges, and for each different proton substitution number. The ^{13}C spectrum and subspectra of the DEPT of the bio-oil samples were divided into similar sections, and the corresponding integral values were adjusted by the appropriate correction factor to compensate for experimental errors arising from incomplete relaxation, polarization transfer, and $^1J_{CH}$ effects. The subspectra CH_1, CH_2, and CH_3 derived from the DEPT analysis appeared to be internally consistent, and provided reliable estimates of the percent CH_n composition for all samples studied (the standard sample mixture and the bio-oils), in agreement with previous studies.[19,20]

An estimate of the percent of unprotonated carbons (CH_0) was obtained by subtracting the integrated peak areas of the DEPT-45 spectrum (final flip angle = 45°) from those of the ^{13}C spectrum. The DEPT-45 spectrum reveals all protonated carbon atoms, but no unprotonated ones, whereas the ^{13}C spectrum displays all carbon atoms, hence the subtraction of their corrected integrated intensities provides an estimate of the unprotonated carbons. This approach assumes that the DEPT-45 spectrum excites all protonated carbons approximately equally. We used two methods for correcting the intensities of these spectra. The first method measured integrated intensities relative to an internal standard (such as TSP, or a sharp isolated peak within the bio-oil); although this gave reasonable results, it required high signal-to-noise ratios for the reference signal, which proved to be difficult to obtain in many bio-oil samples. Instead, we found that similar intensity ratios could be obtained by a second, alternative method in which only the downfield region of the spectrum (above 95 ppm) was assumed to contain unprotonated carbons (CH_0). This downfield region includes aromatic and carbonyl carbons, which are important constituents in the bio-oils. Conversely, bio-oils are expected to contain relatively low concentrations of aliphatic CH_0, which resonate in the upfield region of the spectrum (below ~95 ppm). Thus, the ^{13}C and DEPT-45 spectra were divided into sections according to their chemical shift range. The peaks between 0 and 95 ppm were integrated as a unit (excluding the acetone solvent peak, at 30 ppm), and the integration values of these regions in the ^{13}C and DEPT-45 spectra were set numerically equal to each other, with the other regions scaled correspondingly. By subtracting the overall integration values of the DEPT-45 spectrum from the ^{13}C spectrum (again, excluding acetone solvent peaks at 30 and 207 ppm), an estimate of the integrated intensities for the unprotonated carbons (CH_0) was obtained for resonances greater than 95 ppm.

Comparison of the two analysis methods indicated that they agreed with each other to within 5−10% for those bio-oil samples where the signal-to-noise ratio was sufficient to obtain a reliable integration value of the reference peak. Testing these approaches by analyzing the results from the standard mixture revealed that the percentage of CH_0 groups was reasonably well estimated by both methods[14,18,19] The alternative method estimated that the CH_0 content in the standard mixture was 14.7%, whereas the expected value was 15.8%. Thus, all CH_0 percentages reported here are based on this second method. These experimental estimates will be incorrectly low if there are any unexpected quanternary carbons in the upfield portion of the spectra (0−95 ppm). Therefore, use of this method can only provide a lower estimate of the CH_0 concentration in the actual bio-oils. In addition, the estimates for the bio-oil samples may be adversely affected by the differing relaxation rates (T_1) of protonated and unprotonated carbons.

Results and Discussion

^{1}H NMR Analysis. The proton NMR spectra of the six different bio-oils are shown in Figure 1, and the integral values of selected regions of the spectra on a percentage basis are presented in Table 2. From the spectra it is evident that there are major differences in the overall chemical makeup of the bio-oils from the different feedstocks. The most upfield region of the spectra, from 0.5 to 1.5 ppm, representing aliphatic protons that are attached to carbon atoms at least two bonds removed from a C═C double bond or heteroatom (O or N), was more populated (~30% of all protons) for the bio-oils from chicken litter and guayule than for the others, indicating their higher aliphatic content. For the guayule bio-oil, this high value likely represents pyrolysis products of the natural hydrocarbon rubber and resin components of the guayule plant.[13] By comparison, approximately 20% of the protons contained in the alfalfa stems (legume) bio-oil were from such aliphatic chains, and only ~10% of the protons in the bio-oils from switchgrass and corn stover (herbaceous grass) fell into this category. This suggests that aliphatic hydrocarbon chains, consisting of two bonds or more, are more prevalent in bio-oils following the trend woods > legumes > grasses. This trend also roughly approximates both the trend of carbon content and, consequently, the calorific value of these bio-oils (Table 1).[10−13]

The next integrated region was from 1.5 to 3.0 ppm. This region represents protons on aliphatic carbon atoms that may be bonded to a C═C double bond (aromatic or olefinic) or are two bonds away from a heteroatom. Bio-oils from guayule and chicken litter (mostly wood) again had high levels of protons in this spectra region (~35−45%), but bio-oil from alfalfa stems was the most rich in these protons (54%). The switchgrass and corn stover bio-oils had the least amount of protons in this category (~20%). This suggests that aliphatic portions of molecules, even those bonded to aromatic portions or near heteroatoms, are more prevalent for the higher energy containing bio-oils (Table 1). Water in bio-oil would also likely resonate in this region, but as described in the Methods, the bio-oil collected at the ESP for this analysis had relatively low moisture content. Water content was <2 wt % in bio-oil from guayule and around 10 wt % for the other bio-oils.[10−12] Hence, water signals are not significant features in the spectra.

The next portion of the ^{1}H spectrum, 3.0−4.4 ppm, contained about 10% of the protons in the bio-oils from alfalfa, guayule, and chicken litter and about 20% of the protons in bio-oil from switchgrass and corn stover. This region of the spectrum could represent protons on carbon atoms next to an aliphatic alcohol

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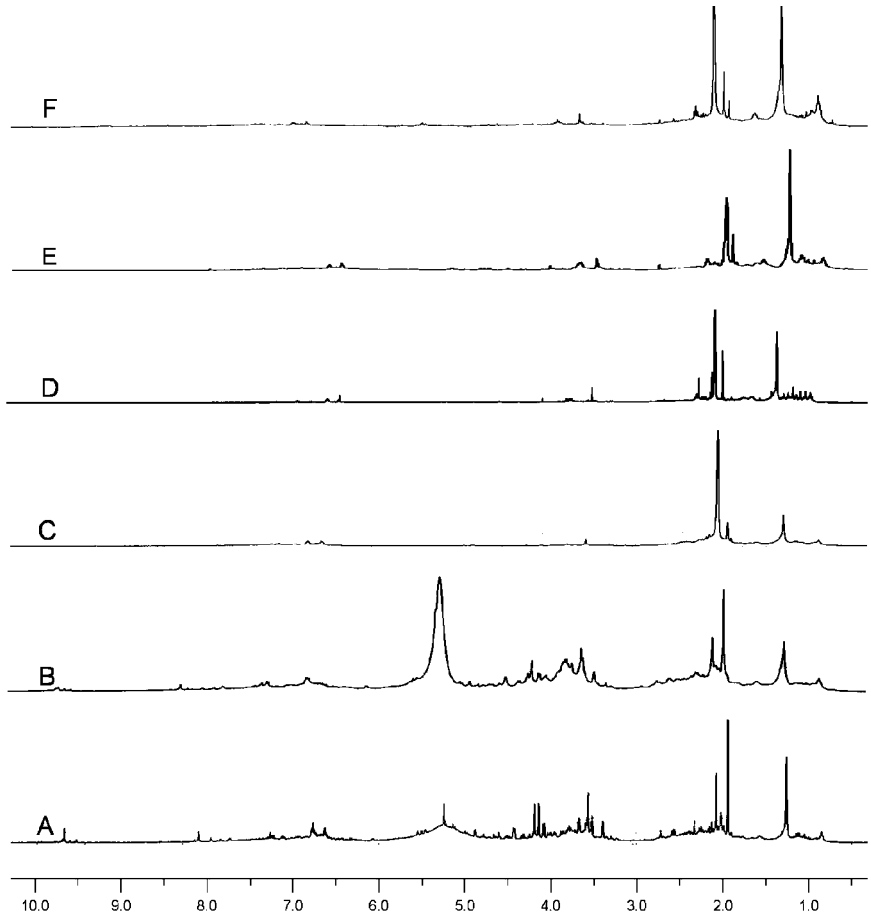

Figure 1. ^{1}H NMR spectra of bio-oils from (A) switchgrass, (B) corn stover, (C) alfalfa stems, (D) guayule (whole shrub), (E) guayule bagasse, and (F) chicken litter.

or ether, or a methylene group that joins two aromatic rings. The later moiety would exist in partially decomposed lignin oligomer present in the bio-oil.

The region between 4.4 and 6.0 ppm represents aromatic ether protons (i.e., lignin derived methoxyphenols) and many of the hydrogen atoms of carbohydrate-like molecules. These protons are most prevalent for bio-oil from herbaceous grass samples, i.e., switchgrass and corn stover (~30%), and are found in smaller amounts in the other bio-oils (2−10%). This is consistent with previous characterizations of these bio-oils that show that levels of the partially dehydrated carbohydrate levoglucosan are high in bio-oil from switchgrass and corn stover and much lower in those from the other feedstocks.[12,13,21]

The aromatic region of the spectrum (6.0−8.5 ppm) contains between 8 and 16% of the protons in the bio-oils. This represents not only those hydrogen atoms in benzenoids, but also those in heteroaromatics containing O and N. Some heteroaromatics resonate above 8.5 ppm, but these are only observed in small quantities in corn stover and switchgrass bio-oils. Interestingly, the amount of aromatic protons does not track with the lignin content of the feedstock (perhaps suggesting that aromatics are created as a result of pyrolysis conditions) nor with the energy content of the bio-oils, although lignin content of the biomass and energy content of the bio-oil often are linked.[1,2] Also, the percentage of aromatic protons for each of the feedstocks is similar for each of the bio-oils, and most of the differences between the bio-oils appear to be from heteroatom content and aliphatic chain growth off of aromatics.

The downfield spectral regions (9.5−10 ppm) of the bio-oils from switchgrass and corn stover contain resonances that most likely arise from aldehydes, although carboxylic acids may also occur in this region. Trace amounts of aldehyde were also detected in the guayule bagasse spectrum, although they are not observeable in the scale presented in Figure 1. No such resonances were detected in the spectra of the other bio-oils.

Overall, the ^{1}H NMR analysis shows that aliphatic protons are most prevalent for those bio-oils derived from wood-like biomasses, while protons proximal to heteroatoms (alcohols, carbohydrates) are highly concentrated in bio-oils from grass-like biomasses.

^{13}C and DEPT NMR Analysis. The ^{13}C NMR spectra of the bio-oils are shown in Figure 2, and integration values of various regions are tabulated in Table 3. Table 3 provides an overview of the entire carbon content within a given chemical shift range, providing information on the types of chemical functional groups that are present in that range, as well as their relative amounts. The percentage values listed in Table 3 for the bio-oil derived from chicken litter agree closely with a previous report on the ^{13}C NMR of bio-oil from chicken litter.[4] The DEPT analyses are summarized in Table 4. This table subdivides the carbon content within each chemical shift range and describes it in terms of the percentage of carbons with different proton substitution numbers. From this information, more structural details may be discerned, such as the extent of branching.

As expected, the bio-oils with a high percentage of carbons resonating in the most upfield region (0−30 ppm) of the ^{13}C spectrum (Table 3) were the same as those with a high percentage of their proton resonances in the upfield region (Table

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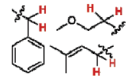
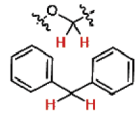
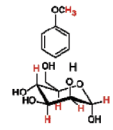
Table 2. Percentage of Hydrogen Based on ^{1}H NMR Analysis of Bio-Oil from Fast Pyrolysis of Various Feedstocks, Grouped According to Chemical Shift Range[a]

Chemical Shifts (ppm)	Proton Assignment	switch-grass	corn stover	alfalfa stems	guayule –whole	guayule bagasse	chicken litter
0.5–1.5	 Alkanes	9.8%	11.8%	20.9%	29.4%	28.7%	34.6%
1.5–3.0	 aliphatics α-to heteroatom or unsaturation	24.3%	18.3%	54.0%	42.0%	34.5%	45.9%
3.0–4.4	 alcohols, methylene-dibenzene	21.3%	20.5%	7.2%	10.4%	12.5%	9.8%
4.4–6.0	 methoxy, carbohydrates	25.7%	30.3%	2.3%	6.8%	9.7%	1.8%
6.0 - 8.5	 (hetero-) aromatics	17.5%	15.1%	15.1%	11.2%	15.6%	7.9%
9.5 - 10.1	 aldehydes	1.3%	1.7%		0.2%	0.5%	

[a] Highlighted atoms are shown in representative chemical functional groups and are intended to only indicate the potential types of chemical environments that may be present. The real mixtures may contain significant structural diversity.

2). However, analysis of the DEPT spectra (Figures 3−8, Table 4) does reveal some differences. The bio-oil obtained from chicken litter had a large percentage of both protons and carbons in the most upfield (aliphatic) regions of the spectra, but only 47% of the carbons in this portion of spectrum were methyl (CH_3) groups. This is the lowest percentage of paraffinic carbons that were found to be methyl groups among the bio-oils studied. Chicken litter bio-oil also has a large percentage CH_1 groups in this spectral region, which can only arise from branch points within an aliphatic chain. The percentage of CH_2 groups in the chicken litter bio-oil in this chemical shift range is also a little higher than the others. Taken together, these percentages suggest that, for the paraffinic portions of the molecules in the bio oil from chicken litter, there may be a somewhat higher proportion of branched and/or cyclic aliphatic structures with medium-short length carbon chains. In corn stover the percentage of methine (CH_1) is similarly high, but that of the methylene (CH_2) is low, also suggesting the presence of branched and/or cyclic structures, in which the chains may be shorter or fewer. Unlike the other bio-oils, virtually all of the methyl groups present in the chicken litter bio-oil were in this upfield region. Lower field methyl groups are present (especially methoxyphenols at ~55 ppm), but most exist in trace amounts and were not easily quantified. This is supported by the small percentage of protons found around 4−5 ppm in the proton spectrum (vide supra) of the bio-oil from chicken litter. For all of the other bio-oils (except

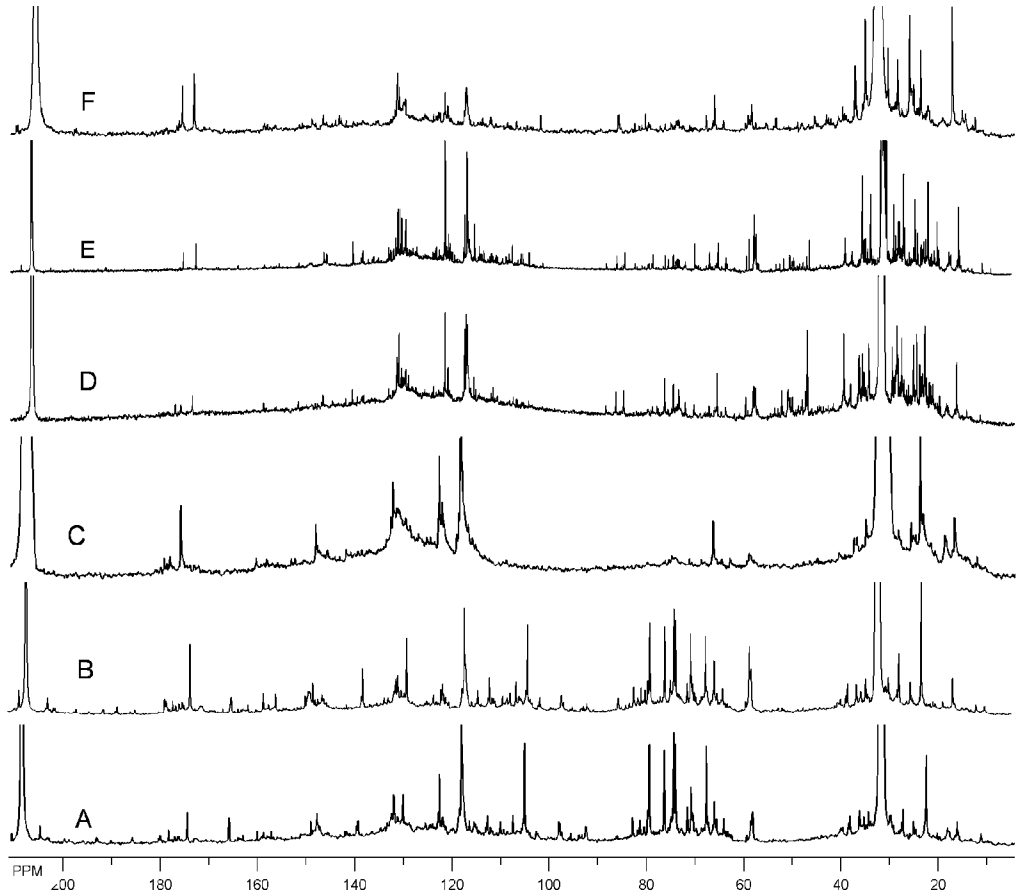


Figure 2. ^{13}C NMR spectra of bio-oils from (A) switchgrass, (B) corn stover, (C) alfalfa stems, (D) guayule (whole shrub), (E) guayule bagasse, (F) chicken litter.

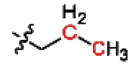
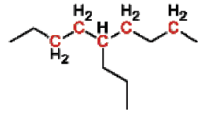
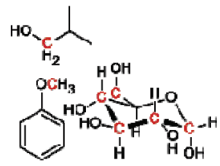
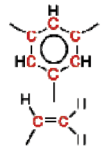
guayule bagasse), more than 70% of the carbons found in the most upfield region (0−28 ppm) of the spectra consisted of methyl groups. The high percentage of nonmethyl carbons in this region of the spectra for the bio-oil from guayule bagasse is likely due to cyclic terpene molecules that are present in the plant resin and their pyrolysis products,[13] which are rich in paraffinic methylene (CH_2) and methine (CH) groups.

The region of the ^{13}C NMR spectrum from 28 to 50 ppm (excluding the acetone solvent) contains a high percentage of the bio-oil carbons. These aliphatic carbon atoms contribute significantly to the energy content (Table 1).[1,2,11] As indicated in Table 3, the feedstocks of guayule and chicken litter produce bio-oils with the highest percentage of carbons that fall in this region (20−30%). A lesser percentage of bio-oil carbon atoms were produced from grasses or legumes (7−12%). For all of the bio-oils in this study, this spectral region is dominated by methylene carbons (CH_2) (Table 4), and they are assigned as part of long aliphatic chains, although the corn stover bio-oil contains the least, with only 40%.

This region (28−50 ppm) can be viewed as a continuation of the previous aliphatic region (1−28 ppm), as most of the carbon atoms found here resonate upfield, closer to 30 ppm. For example, methylene carbons that are more than ca. two bonds from the terminus of an alkane chain, or which are adjacent to branch points, tend to occur above ∼28 ppm, but below ∼40 ppm, so these resonances can imply the presence of longer aliphatic chains. More information may be obtained by the DEPT analysis, as it provides the relative amounts of CH_2 and CH_3, which is an indication of the lengths of the chains. Similarly, a high percentage of CH_1 points toward a high degree of branching within the chains. In the case of corn stover bio-oil, Table 4 indicates that ∼60% of the carbons in the 28−50 ppm region are methines (CH_1), suggesting that corn stover bio-oil contains more highly branched aliphatic groups than in the other bio-oils. The total percentage of such highly branched chains is low, however, as they only comprise 10% of the total carbons in this bio-oil (Table 3). This is notably different from that of the other herbaceous feedstock, switchgrass, which has a similar total carbon content in this spectral range, but only ∼5% consists of methines. Thus, switchgrass contains mostly linear aliphatics. By comparison, the bio-oil from guayule bagasse also had a significant amount of methine (CH_1) content in this region, but methylenes (CH_2) were still the majority in this case. This spectral region may also contain resonances from allylic or benzylic carbons, which may contribute some intensity to the CH_1 and CH_2 DEPT subspectra. Likewise, carbons adjacent to C═O could be observed here, but these are not expected to occur in detectable amounts. In this chemical shift range, the only methyl groups that were found in significant quantities were in the bio-oil from whole plant guayule, where ∼15% of the protonated carbons in this region were methyl groups. Nearly all of this methyl intensity was associated with a single peak at 42 ppm, but no assignment could be made.

A review of the entire aliphatic region (0−55 ppm) can also be informative. Approximately 50% of the carbons in the guayule and chicken litter bio-oils are found in this region, compared to ∼20−30% for the other oils. If it is assumed that there are no quaternary carbons in this region, then the information in Tables 3 and 4 may be combined. This reveals that the bio-oils from chicken litter and guayule (whole and bagasse) have the highest total aliphatic CH_2 content (∼20−25% of their total carbon), followed by the bio-oils from alfalfa stems (∼15%) and switchgrass (∼10%), with corn stover bio-oil having the least CH_2 content (4%). These percentages relate to

Table 3. Percentage of Carbon Based on ^{13}C NMR Analysis of Bio-Oil from Fast Pyrolysis of Various Feedstocks, Grouped According to Chemical Shift Range[a]

Chemical Shifts (ppm)	Carbon assignments[b]	switch-grass	corn stover	alfalfa stems	guayule –whole	guayule bagasse	chicken litter
0–28	 short aliphatics	13.8%	13.8%	17.2%	28.5%	19.1%	25.8%
28–55	 long and branched aliphatics	7.3%	10.3%	12.2%	24.4%	29.0%	21.8%
0-55 Total	All of above	21.1%	24.1%	29.4%	52.9%	48.1%	47.6%
55–95	 alcohols, ethers, phenolic-methoxys, carbohydrates sugars	24.7%	30.8%	16.1%	6.7%	7.7%	13.6%
95-165	 aromatics, olefins	53.0%	36.0%	51.9%	39.5%	43.5%	36.2%
165-180	 esters, carboxylic acids		3.8%	2.6%	0.8%	0.4%	
180-215	 ketones, aldehydes	1.2%	1.5%		0.1%	0.2%	2.6%

[a] The strong acetone solvent resonances at 30 ppm (CH_3) and 207 ppm (C═O) were excluded from this analysis. [b] Highlighted atoms are shown in representative chemical functional groups and are intended to only indicate the potential types of chemical environments that may be present. The real mixtures may contain significant structural diversity.

the lengths and concentrations of the aliphatic chains. The extent of branching can be implied from CH_1 percentages, for which the chicken litter bio-oil has the highest content (14%), followed by guayule bagasse and corn stover (11% and 9%, respectively). These numbers would be smaller if quaternary carbons are present, but both types of carbons indicate the presence of a small amount of branched aliphatic chains. The $CH:CH_2:CH_3$ ratios found in the corn stover bio-oil are consistent with aliphatic chains (linear or cyclic) with many methyl groups branching off, since the percent of CH is approximately equal to the percent of CH_3.

The region between 55 and 95 ppm in the ^{13}C NMR spectra represents carbon atoms adjacent to an O atom in carbohydrates, ethers, or alcohols. Also, carbon atoms adjacent to nitrogen

Table 4. Percentage of Carbon Atoms as CH_0, CH_1, CH_2, and CH_3 for Various Spectral Regions Based on ^{13}C and DEPT NMR Spectra, Grouped According to Chemical Shift Range[a]

chemical shifts (ppm)		switchgrass	alfalfa stems	corn stover	guayule (whole)	guayule bagasse	chicken litter
0−30	CH_1			22		4.5	19
	CH_2	24	30	2	24	34	33
	CH_3	76	70	77	76	62	47
30−48	CH_1	5	15	60	15	35	43
	CH_2	87	85	40	70	65	57
	CH_3	8			16		
48−90	CH_1	51	50	85	63	67	100
	CH_2	40	48	6	28	22	
	CH_3	9	2	7	8	11	
90−165	CH_0	68	52	73	72	68	73
	CH_1	29	48	27	26	32	27
	CH_2	3			2	<1	
	CH_3						
165−180	CH_0		100	100	100	100	
180−215	CH_0	∼100		87	100	∼100	100
	CH_1	observed[b]		13		observed[b]	
Total	CH_0	37	27	28	29	30	29
	CH_1	28	35	45	18	30	38
	CH_2	21	23	6	27	27	21
	CH_3	14	13	13	26	13	12

[a] The strong acetone solvent resonances at 30 ppm (CH_3) and 207 ppm (C═O) were excluded from this analysis. [b] Weak peaks and low signal-to-noise ratios in this spectral region made these integration values unreliable.

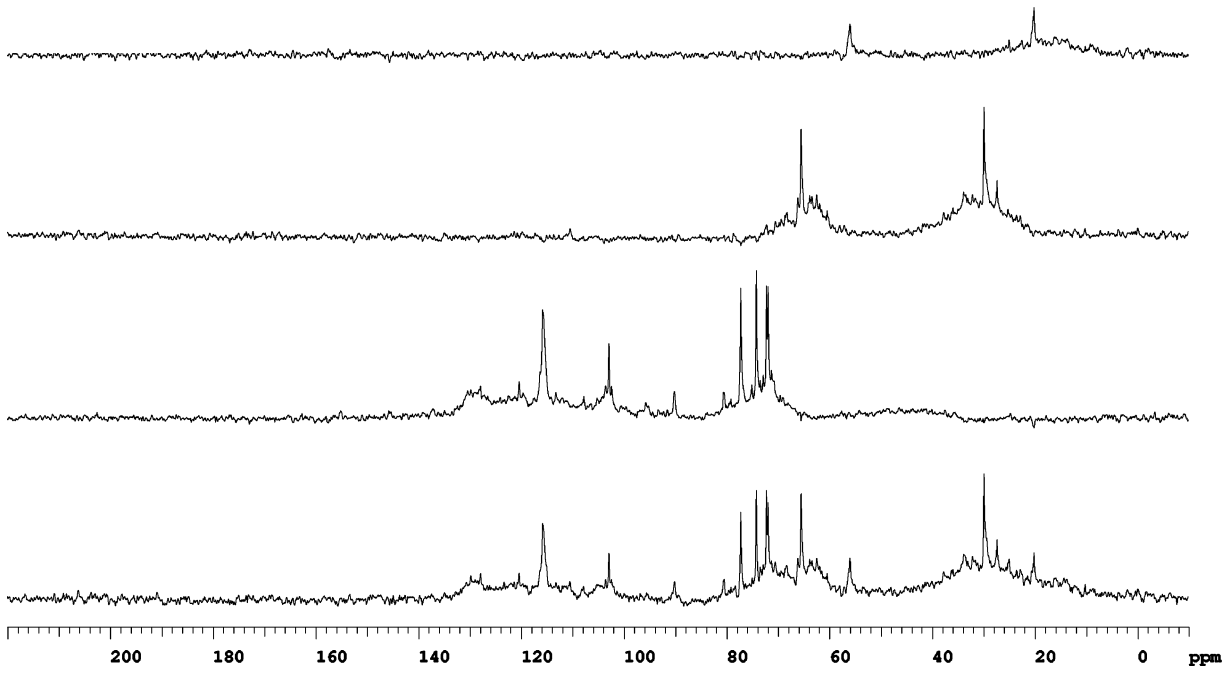


Figure 3. DEPT spectra of switchgrass bio-oil: (bottom) all protonated C, (second from bottom) CH, (third) CH_2, and (top) CH_3.

atoms would resonate in this region. Predictably, in this chemical shift range, the relative amount of these types of carbon atoms follows the same general trend as their overall oxygen and nitrogen content: corn stover ∼ switchgrass > alfalfa ∼ chicken litter > guayule (Table 1).[10−13] This carbon region gives complimentary information to that observed in the ^1H spectra (4.4−6 ppm). The bio-oils from herbaceous samples (corn stover and switchgrass) have the largest CH content in this region due to the presence of partially dehydrated sugars (e.g., levoglucosan). Whereas in the ^1H spectra it was not possible to distinguish between the carbohydrate resonances and those from other sources, such as methoxy groups, this determination is simpler with the DEPT analysis. The carbohydrate component is observed as the primary source of signal within this chemical shift range of the CH subspectra of the DEPT experiment. These methine carbons comprise 85% of the carbons in this region of the corn stover bio-oil spectra and 51% of that of switchgrass. While the DEPT analysis of the chicken litter bio-oil detected neither CH_2 nor CH_3 carbons in this region, but only CH, this region of the DEPT subspectrum was relatively unstructured and uninterpretable. Levoglucosan also has an CH_2 that should be observed in this region, but it was not uniquely identifiable. For all of the bio-oils studied, the only methyl groups found in this region were observed at ∼55 ppm in all cases and are the result of methoxy groups on phenolics (i.e., guiaicol and syringol dervivatives), often found in lignin and its pyrolysis products.[1,2] Although these methoxy groups exist in relatively low overall concentration (<3% of the total carbon content), they may be important considerations for potential end products made from bio-oil.

The region between 95 and 165 ppm in the spectrum represents the aromatic portion of the bio-oils. In addition to benzenoids, carbon atoms in heteroaromatics that contain O or N will also resonate in this region, as will any olefinic carbons. Overall, the aromatic carbon content ranged between 36 and 53% of all carbon atoms in the bio-oils. As was the case in the

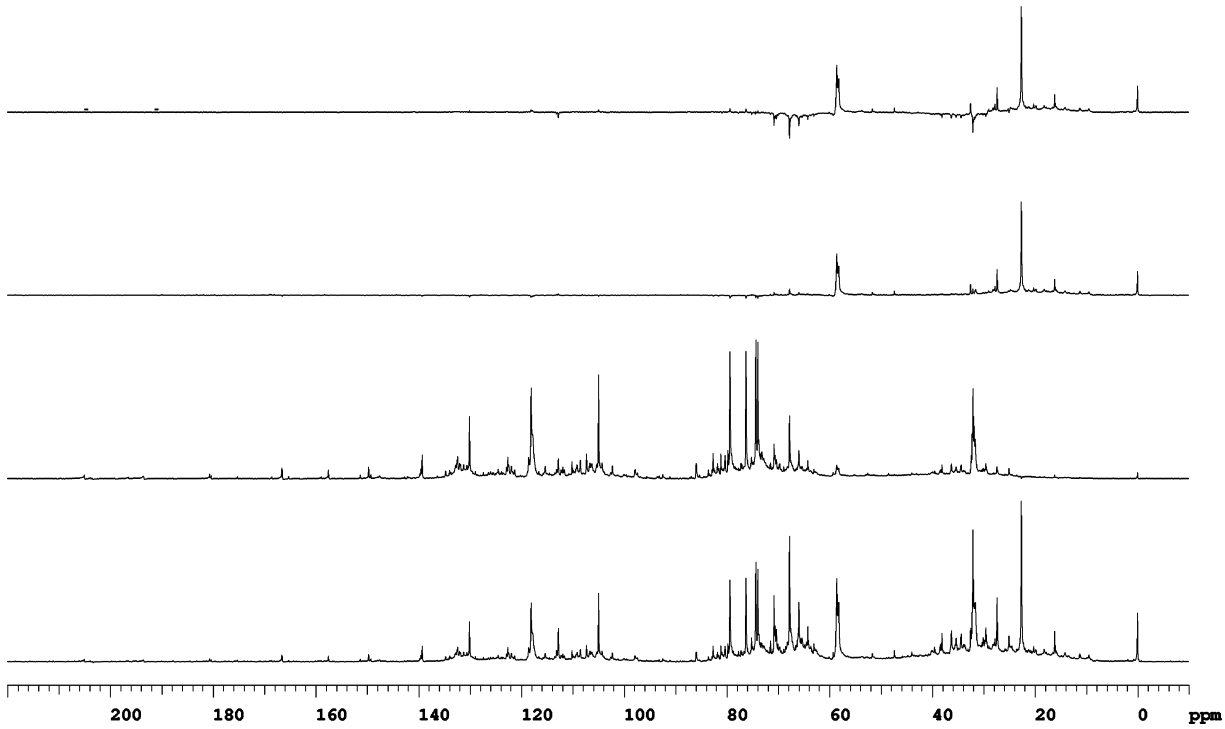


Figure 4. DEPT spectra of corn stover bio-oil: (bottom) all protonated C, (second from bottom) CH, (third) CH_2, and (top) CH_3.

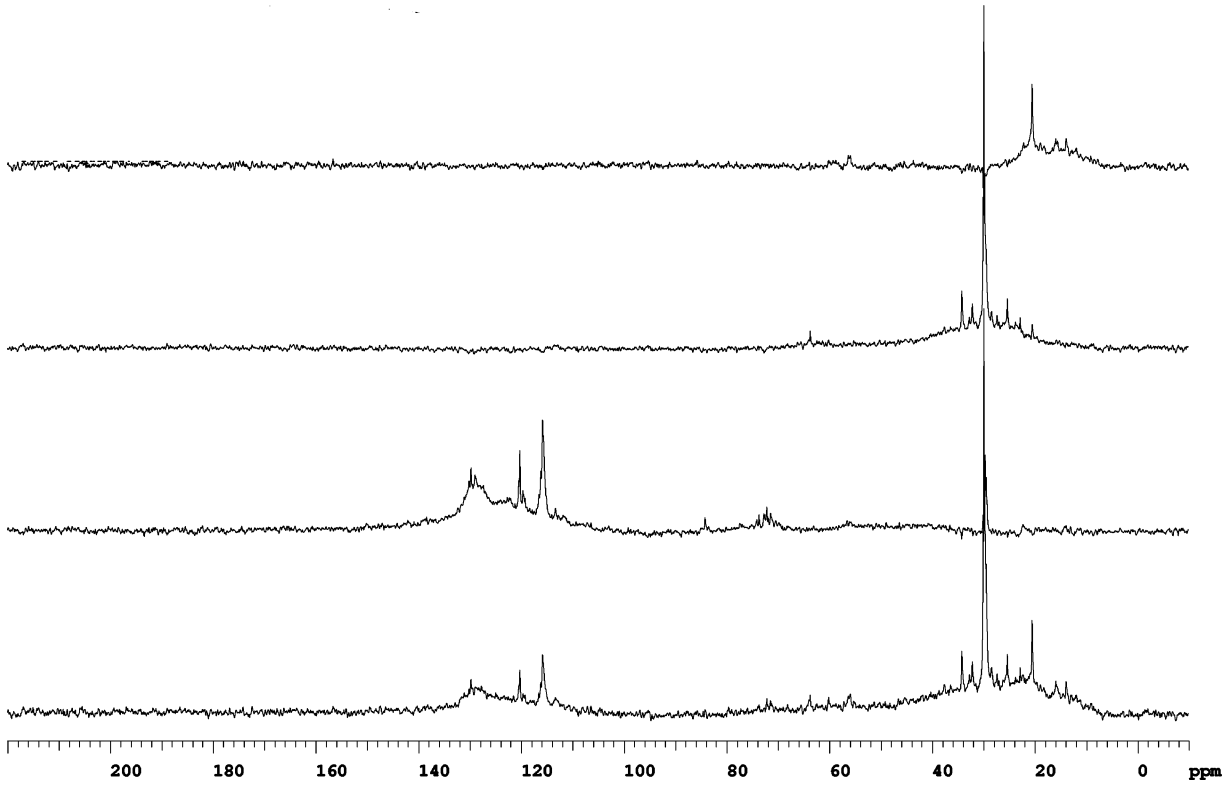


Figure 5. DEPT spectra of alfalfa stems bio-oil: (bottom) all protonated C, (second from bottom) CH, (third) CH_2, and (top) CH_3.

^{1}H NMR spectra, the relative amount of aromatic carbon atoms in the bio-oil did not reflect the relative lignin content of the feedstock from which the bio-oil was derived.[10−13] Several explanations may be proposed for this observation. Lignin may be fractionally converted to char, as it is more likely to due so compared to cellulose or hemicellulose, under these conditions. Alternatively, it may be that the pyrolysis conditions convert nonaromatic moieties into aromatic ones.

Aromatic carbon content of the bio-oils followed the trend switchgrass ∼ alfalfa > guayule > chicken litter ∼ corn stover (Table 3). Not surprisingly, the DEPT spectra of this region consists nearly exclusively of CH_1. The bio-oils from switchgrass and guayule contain small amounts of CH_2 found in this region, which may arise from molecules containing terminal alkenes.

The CH_0 content in these bio-oil samples was assumed to mostly derive from substituted positions on aromatic compounds, rather than from aliphatic branching. This assumption appears to be justified given that the aliphatic CH_1 percentages indicate relatively low amounts of branching, although the

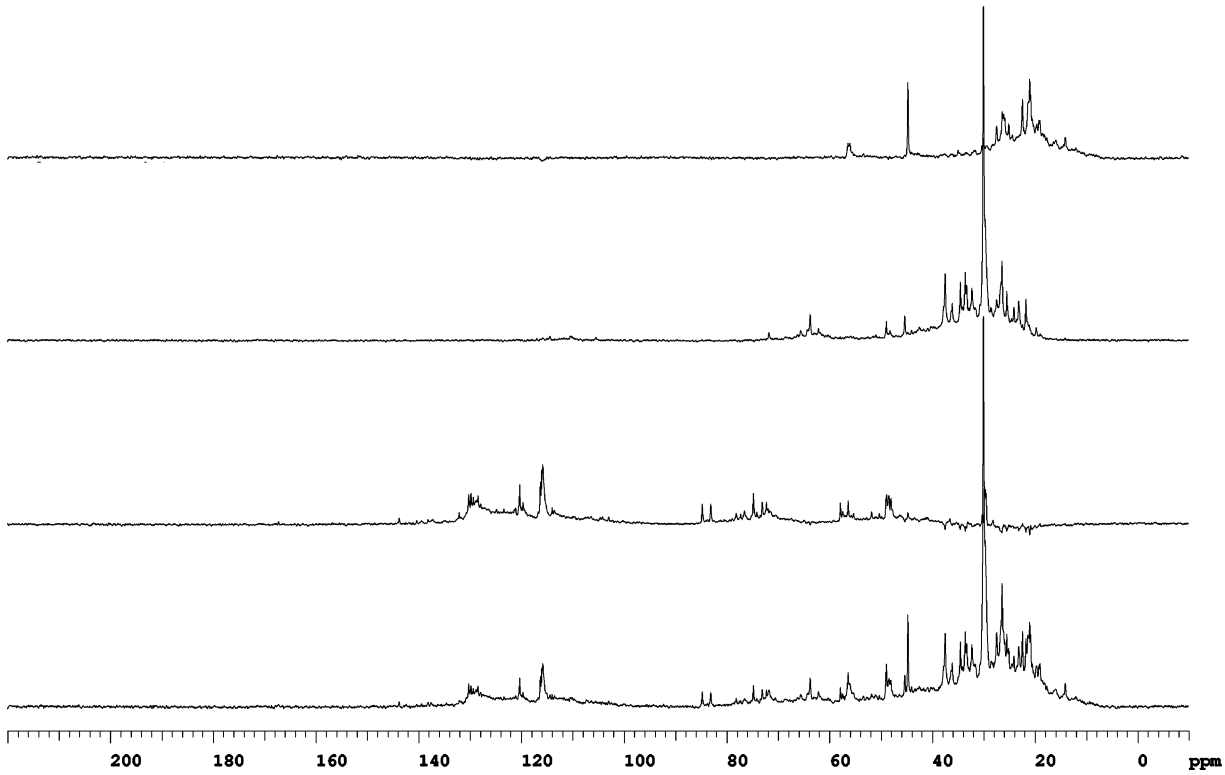


Figure 6. DEPT spectra of whole guayule bio-oil: (bottom) all protonated C, (second from bottom) CH, (third) CH_2, and (top) CH_3.

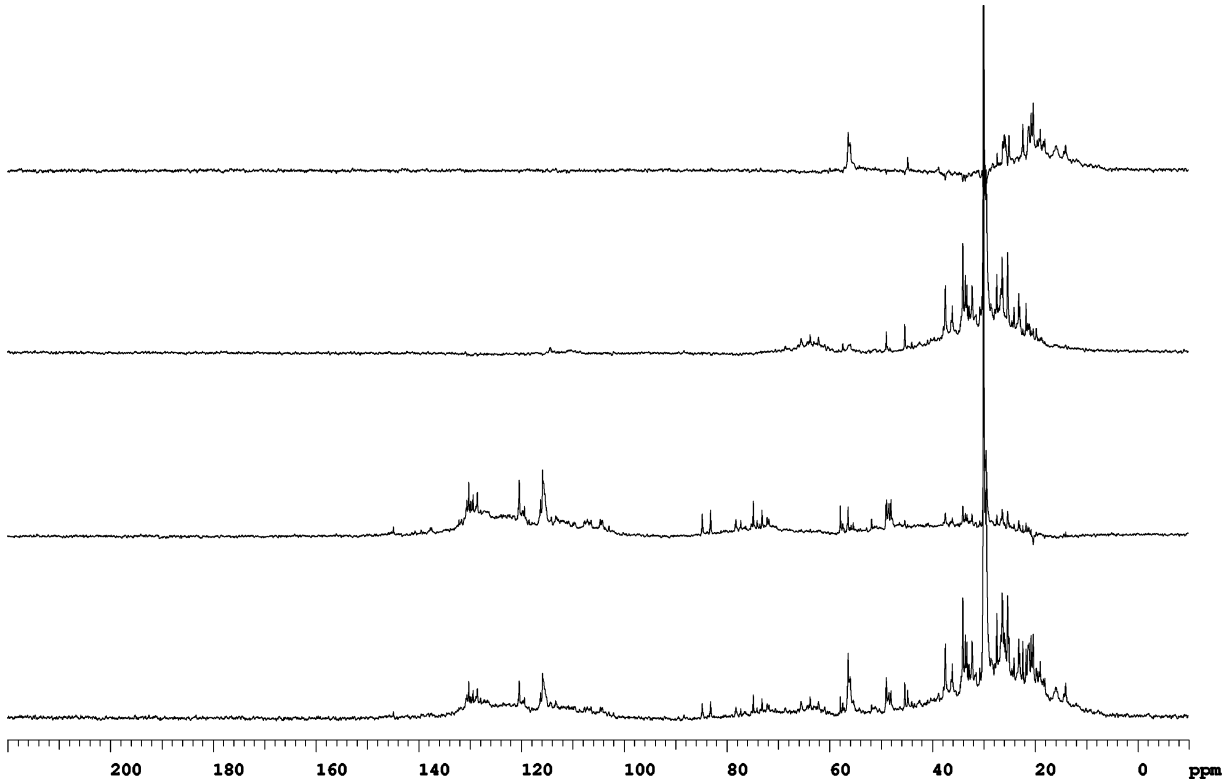


Figure 7. DEPT spectra of guayule bagasse bio-oil: (bottom) all protonated C, (second from bottom) CH, (third) CH_2, and (top) CH_3.

percentages of CH_1 and CH_0 are not necessarily correlated. Thus, the CH_0 content in the aromatic region was estimated by subtracting the integral values of the DEPT-45 from that of ^{13}C spectra over the range of 95−165 ppm using the approach described in the Methods. According to this analysis, in most cases, the aromatic CH_0 carbons outnumbered CH_1 carbons by >2:1. The exception to this was bio-oil from alfalfa stems, where the aromatic CH_0:CH_1 ratio was nearly 1:1. This may be a reflection of the lignin structure of the feedstock, or it may suggest that the C−C and C−O bonds connecting substiutuents to aromatic rings were broken more frequently during the pyrolysis of alfalfa stems than is the case with the other feedstocks. If correct, the high CH_0:CH_1 ratio also suggests that aromatic molecules in the bio-oil are quite complex, with most benzene rings having four or more positions substituted, and also suggests that simple aromatics and simple phenols (those

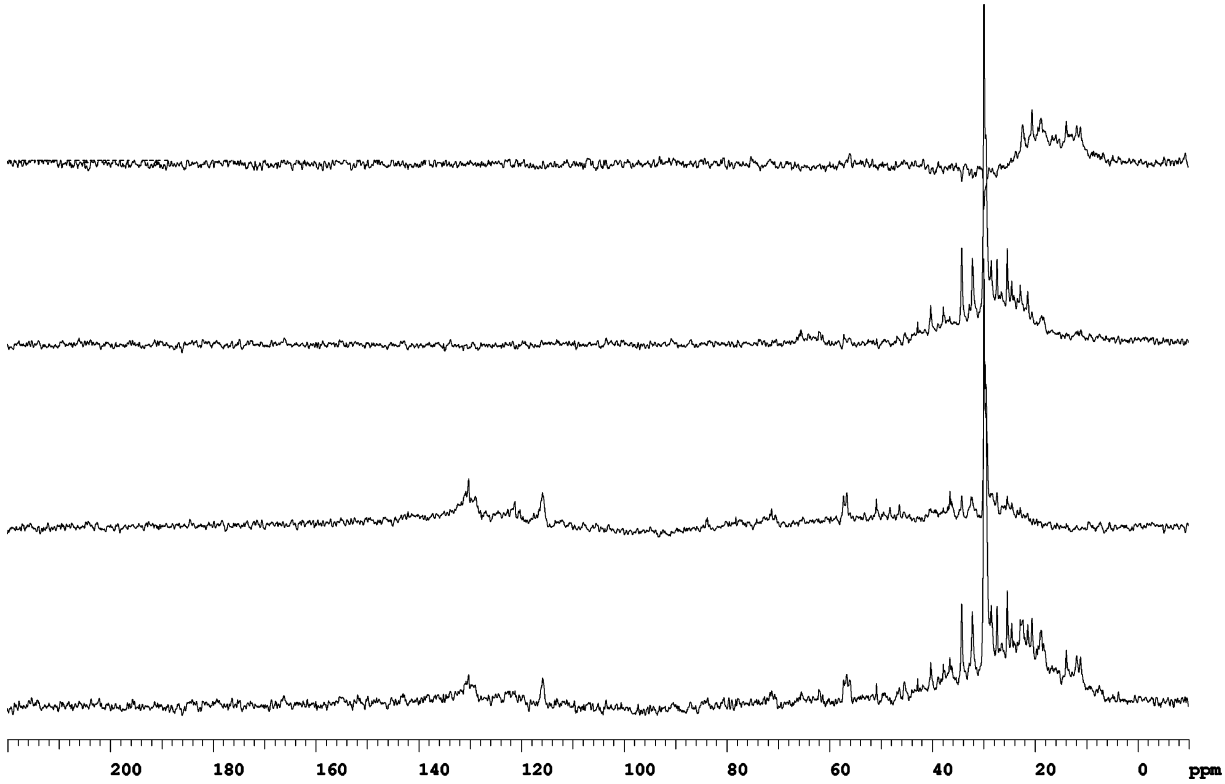


Figure 8. DEPT spectra of chicken litter bio-oil: (bottom) all protonated C, (second from bottom) CH, (third) CH_2, and (top) CH_3.

low ring substitution numbers) are not prevalent in the ESP fraction of theses bio-oils. Previous HPLC and GC analyses[12,13,21] have not observed this large percentage of complex aromatics. It is likely that their complexity makes them difficult to extract or volatilize and hence are not detected by these methods. The ability of NMR to reveal them is, therefore, significant. Carbonyl carbon atoms are found in the most downfield regions of the ^{13}C spectra. Acid and ester carbonyl carbons are found in the 165−180 ppm range, while ketone and aldehyde carbons are found farther downfield, above 180 ppm. Esters and acids were most prevalent in the bio-oils from corn stover and alfalfa. Chicken litter bio-oil had the highest percentage of its carbons assigned as ketones (CH_0), followed by the bio-oils from corn stover and switchgrass (Table 3). The bio-oils from corn stover, switchgrass, and guayule bagasse had aldehyde (CH_1) concentrations that were somewhat higher than the other bio-oils (Tables 3 and 4), whereas the other bio-oils did not have detectable levels of aldehydes in their DEPT subspectra. Only the DEPT spectra for the corn stover bio-oil had sufficient signal-to-noise to make any quantitative estimates for these functional groups. By combining Tables 3 and 4 for the corn stover bio-oil, it is estimated that ketones comprise about 1.5% of its total carbon content, whereas aldehydes comprise ∼0.3% of its total carbon content. The alfalfa stem bio-oil had only detectable levels of esters and acids (2.6% of total carbon) but no detectable amounts of ketone or aldehyde. The other bio-oils appeared to contain predominantly ketones, rather than aldehydes.

Conclusions

^1H, ^{13}C, and DEPT NMR spectroscopy were used to characterize fast-pyrolysis bio-oils from six different feedstocks, including woodlike, legume, and grasslike biomasses. In addition to classifying hydrogen and carbon atoms based on chemical shift, using DEPT analysis allowed for further classification of carbon atoms based on the number of attached protons. The following are among the numerous differences in the composition of the bio-oils that were evident from the analysis.

(1) The relative number of aliphatic carbons not adjacent to heteroatoms (represented by those carbon atoms and protons that resonate in the upfield regions of the spectra) tracked with the dry-basis energy content of spectra (Table 1). Aromatic carbon or proton content did not track in the same manner.

(2) Bio-oil from chicken litter had the lowest overall amount of methyl groups of the bio-oils studied.

(3) The ^{13}C and DEPT analyses indicate that, among the bio-oils studied, those derived from corn stover and switchgrass had the fewest aliphatic carbons. The large percentage of methine (CH_1) groups in the corn stover bio-oil suggests that its aliphatics were highly branched. Since the percentage of methyl groups in corn stover is nearly the same as that of its CH_1, while its percentage of CH_2 is low, it is surmised that these branches are very short, consisting mostly of methyl groups. Conversely, bio-oil from switchgrass appears to have more straight-chain aliphatics.

(4) Intact carbohydrate content was very high in bio-oil from corn stover, as a high concentration of protons was found at ∼5 ppm in the ^1H NMR and a high concentration of CH_1 carbons was found between 60 and 80 ppm. The DEPT analysis more easily distinguished these resonances from those of overlapping functional groups and allowed more accurate quantitation. For example, ethers, especially methoxy groups, were readily observed in a DEPT spectra, and although their concentrations are low, their presence may represent an important consideration for some end products.

(5) The aromatic region generally had CH_0:CH_1 ratios of >2:1, suggesting that aromatics found in the bio-oil are highly complex, with the average benzene ring having at least four substituents. Thus, simple aromatics and simple phenols are not prevalent in these ESP fractions of bio-oils. Since unsubstituted

aromatic carbons (CH) are potentially important reactive sites for synthetic modification, the aromatic CH_0:CH_1 ratio may be an important consideration when evaluating the suitability of a feedstock for end product derivitization. As determined from Table 4, the aromatic CH_0:CH_1 ratio for the bio-oil from alfalfa stems was the lowest (1:1), while guayule bagasse and switchgrass resulted in bio-oils that had similar ratios (∼2.1−2.3), and corn stover, chicken litter, and whole plant guayule appeared to have the highest ratios (∼2.7−2.8).

(6) Detecting relative aldehyde or ketone concentrations may be useful information for applications involving the synthetic modification of bio-oils. All spectroscopic methods employed in this work indicate that switchgrass, corn stover, and guayule bagasse bio-oils have detectable levels of aldehydes. Chicken litter bio-oil has the highest ketone content, although they are observed in all of the bio-oils except the alfalfa stem bio-oil, which had mostly esters or acids.

These analyses demonstrate that quantitation of the ^{1}H, ^{13}C, and DEPT experiments can provide important information about not only the kinds of chemicals in bio-oils but also their relative concentrations. This approach can reveal whether the alkanes in a bio-oil consist of predomininantly long or short chains and the extent of branching within them, potentially useful information for further refining of bio-oils into particular types of liquid transportation fuels. Additionally, it provides information on the concentrations of chemical functionalities that are potentially useful for synthetic modifications and hence may help to guide those interested in using bio-oil as a chemical feedstock. Using NMR also provided indications of highly substituted aromatic groups, which were not detected by other means, indicating a lost mass problem that needs to be addressed by users of GC and HPLC.

The efficiency of future studies may be improved by reducing the duration of the experimental time by means of relaxation agents and by the use of greater magnetic field strengths to improve signal sensitivity.

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